Figure S1. **Roles of caveolae and dynamin in *E. coli* invasion.** (A) Caveolae are portals for the *E. coli* invasion into BECs. The cholesterol sequesterer methyl-β-cyclodextrin (MβCD) dose-dependently (1 and 2 µM) decreased the *E. coli* ORN103(pSH2) invasion of BECs, whereas subsequent readdition of cholesterol restored the bacterial invasion. Data are expressed relative to cells not treated with MβCD or cholesterol. n = 3; *, P < 0.01 versus MβCD control. (B) Forced overexpression of wild-type or K44A dynamin2 does not affect the binding of UPEC to BECs. *E. coli* ORN103(pSH2) binding to BECs was assessed at 4°C, and no significant difference was seen in wild-type (WT) or K44A dynamin2-overexpressing cells compared with control empty vector (EV)-transfected BECs. Data are expressed relative to empty vector cells. n = 3. (C) Dynamin2 does not impact the invasion of *S. typhimurium*. Overexpression of wild-type or K44A dynamin2 does not affect the entry of an invasive strain of *S. typhimurium* into BECs. Data are expressed relative to control empty vector-expressing cells. n = 5. (D) Stable dynamin2 knockdown in BECs. Cells were infected with lentivirus encoding control shRNA that targets GFP (GFPshRNA) or shRNAs that target dynamin2 (Dyn2shRNA-6649, Dyn2shRNA-6650, or Dyn2shRNA-6651). Equal amounts of cell lysate on nitrocellulose filters were immunoblotted with dynamin2 (top) and GAPDH (bottom) antibodies. Data represent means ± SEM.
**Figure S2. Incubation of BECs with bacteria promotes NO formation.** (A) BECs were incubated with *E. coli* ORN103(pSH2) for the indicated times. NOSs convert l-arginine to l-citrulline in a reaction that forms NO as a byproduct and can be experimentally measured by assessing the formation of [3H]-citrulline from [3H]-arginine. BECs were incubated with [3H]-arginine alone or together with *E. coli* ORN103(pSH2). Cells were lysed at the indicated times, and equal amounts of protein were added to a column to separate [3H]-citrulline. The flow-through fraction was analyzed by liquid scintillation. (B) Real-time detection of NO in live BECs. Representative images of a time-dependent (in minutes) conversion of the NO-reactive probe DAF-2DA (not fluorescent) to DAF-2T (fluorescent). Images were acquired after cells were loaded with DAF-2DA (in minutes) and are representative of results obtained from three independent experiments. Numbers at the top reflect the duration of treatment (in minutes). NT, not treated.

**Figure S3. Knockdown of dynamin2 and eNOS attenuates UPEC invasion.** (A) shRNA-mediated reduction of dynamin2 and eNOS protein expression in BECs. (B and C) Bacterial adherence (B) and bacterial invasion (C) after exposure of BECs expressing GFPshRNA, Dyn2shRNA, eNOSshRNA, or Dyn2/eNOSshRNA to CFT073, UT189, or UT189ΔFimH. For CFT073, data are shown relative to GFPshRNA-expressing cells. For UT189 and UT189ΔFimH, data are shown relative to GFPshRNA-expressing cells that were incubated with UT189. n = 3; *, P < 0.05 versus values from corresponding GFPshRNA-transfected samples. FimH, UT189ΔFimH. Data represent means ± SEM.
Figure S4. Dynamin2 S-nitrosylation is required for UPEC entry into BECs. (A) Acid wash is efficient to remove membrane-bound UPEC. BECs were starved, prechilled on ice, and infected with HcRed-expressing E. coli on ice for 15 min. The cells were divided into three groups. For group 1 (a–d), cells were fixed immediately after infection. For group 2 (e–h), cells were washed with PBS, incubated in culture medium for 1 h (to allow bacterial invasion), washed with acid solution, and fixed. For group 3 (i–l), cells were sequentially washed with PBS and acid solution, incubated in culture medium for 1 h, and then fixed. Higher magnification of the insets in c, g, and k are shown in d, h, and l. Actin was stained with Alexa Fluor 488 phalloidin. (B) BECs were transiently transfected with cDNAs encoding GFP-dynamin2 (wild-type [WT], K44A, C86A, C607A, or C86/607A) and subjected to invasion assay with HcRed-expressing E. coli. Cells were washed with acid solution, fixed, and processed for immunofluorescence. For each transfectant, 100 GFP-expressing cells were randomly selected, and the number of internalized HcRed-expressing E. coli was counted. Data are expressed relative to control GFP-expressing cells. n = 3; *, P < 0.05 versus control values. Data represent means ± SEM.
Figure S5. UPEC invasion leads to dynamin2 enrichment on the plasma membrane. (A) Intracellular distribution of HA-dynamin2. BECs were transfected with HA-dynamin2 and processed for immunofluorescence using anti-HA antibody. (A, a) Overexpressed HA-dynamin2 was observed in punctated structures and on the plasma membrane. (A, b–d) BECs were transfected with HA-dynamin2, infected with HcRed-expressing *E. coli*, fixed, and then processed for immunofluorescence imaging after staining with anti-HA antibody. The representative images show enrichment of HA-dynamin2 at sites of bacterial entry and attachment (d, arrows). (B) BECs overexpressing HA-dynamin2 (wild-type [WT] or C86/607 mutated form) were infected or not infected with *E. coli*. Plasma membranes were isolated by differential centrifugation, and proteins were resolved by SDS-PAGE followed by Western blotting with anti-HA antibody. Actin was used as a loading control.